

A PROVISIONAL STUDY OF NORTHERN BLUEFIN TUNA POPULATIONS

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SUMMARY

In recent years, there has been considerable debate about whether northern Atlantic bluefin tuna exist as a panmictic unit. This question has been addressed by examining the mitochondrial DNA control region sequences of 140 individuals of northern bluefin tuna from the Mediterranean Sea and the western Atlantic Ocean. No differences were detected either between localities within the Mediterranean Sea or between two different sampling years within one locality. However, a comparison of the distribution of mtDNA lineages between the pooled Mediterranean data and a sample from the western North Atlantic rejected the null hypothesis of panmixis. These results were supported by statistically significant values obtained with both hierarchical analysis of nucleotide diversity and with Chi-square analysis using Monte Carlo randomizations. The departure from homogeneity is primarily due to the distribution of haplotypes belonging to a well supported monophyletic group of mtDNA lineages that occurred at a frequency of 0.11 in the Atlantic sample and about 0.31 in the Mediterranean sample. These preliminary data suggest that allele frequency differences between western Atlantic and Mediterranean bluefin tuna samples exist. However, extensive sampling will be needed to confirm the existence of defined sub-populations and to define their geographic distribution.

RÉSUMÉ

Des délibérations prolongées ont porté ces dernières années sur la question de savoir si le thon rouge de l'Atlantique Nord constituait une unité panmictique. Ceci a été abordé en examinant les séquences de la zone de contrôle de l'ADN mitochondrial chez 140 thons rouges en provenance de la Méditerranée et de l'Atlantique Ouest. Aucune différence n'a été détectée, ni au sein de la Méditerranée, ni entre les années échantillonnées dans un même endroit. Toutefois, une comparaison de la distribution des lignages de l'ADNmt entre les données méditerranéennes regroupées et un échantillon de la partie occidentale de l'Atlantique Nord contredisait l'hypothèse panmictique nulle. Ces résultats sont étayés par des valeurs statistiques significatives obtenues à la fois par l'analyse hiérarchique de la diversité des nucléotides et avec une analyse des carrés Chi utilisant une distribution aléatoire de type Monte-Carlo. L'homogénéité est surtout mise en question par la distribution des haplotypes appartenant à un groupe monophylétique bien défini de lignages de l'ADNmt qui se présentent à une fréquence 0.11 dans l'échantillon atlantique et à environ 0.31 dans l'échantillon méditerranéen. Ces données préliminaires suggéreraient qu'il existe en effet des différences quant à la fréquences des allèles entre le thon rouge de l'Atlantique Ouest et celui de la Méditerranée. Toutefois, il faudra effectuer un échantillonnage intensif pour confirmer l'existence de sous-populations concrètes, et définir leur aire de répartition géographique.

RESUMEN

En los últimos años, ha habido un considerable debate sobre si el atún rojo del Atlántico norte constituía una unidad panmítica. Esta cuestión se ha tratado examinando las secuencias de la región de control del ADN mitocondrial de 140 ejemplares de atún rojo del norte procedentes del Mediterráneo y del Atlántico oeste. No se detectaron diferencias entre las localidades dentro del Mediterráneo ni entre muestras de diferentes años dentro de una misma localidad. Pero una comparación de la distribución de los linajes del ADNmt entre los datos mediterráneos agregados y una muestra del Atlántico noroeste, eliminaba la hipótesis nula de panmixia. Estos resultados estaban apoyados por valores estadísticos significativos obtenidos tanto por análisis jerárquico de diversidad nucleotídica como por análisis ji-cuadrado, usando técnicas aleatorias Montecarlo. La falta de homogeneidad se debe sobre todo a la distribución de haplotipos pertenecientes a un grupo monofilético de linajes de ADNmt con buena base que ocurrían con una frecuencia de 0.11 en la muestra del Atlántico y de alrededor de 0.31 en la muestra del Mediterráneo. Estos datos preliminares sugieren que existen diferencias de frecuencias de alelos entre las muestras de atún rojo del Atlántico oeste y del Mediterráneo. No obstante, será necesario realizar un amplio muestreo para confirmar la existencia de subpoblaciones definidas y para establecer su distribución geográfica.

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1. INTRODUCTION

The Atlantic northern bluefin tuna are currently managed by ICCAT as two units, one located in the west-Atlantic and the other in the east Atlantic and the Mediterranean Sea. This division is partly based on the existence of two spawning areas, one in the Mediterranean Sea and a second in the Gulf of Mexico. For management purposes, it is assumed that fisheries in the western Atlantic are predominately west Atlantic fish and that fisheries in the eastern Atlantic are predominately east Atlantic fish. However, a recent interpretation of tagging data has suggested that trans-Atlantic migration may be occurring at levels sufficiently high that the Atlantic northern bluefin tuna should be considered a single population unit (National Research Council, 1994).

One approach to test the hypothesis of a single population unit would be a genetic analysis. To stimulate genetic analyses of bluefin tuna, a workshop was convened in Charleston, SC in 1994 that resulted in a well-defined plan (Dean and Woodley, 1994; Ahlquist, 1998). The primary hypothesis to be tested was that Atlantic bluefin tuna consist of a single genetically homogenous population. We present here *preliminary results* from one approach for testing this hypothesis. Nucleotide sequence of the mitochondrial DNA control region from bluefin tuna samples obtained from the northwestern Atlantic and the Mediterranean Sea were compared. Our analyses of these nucleotide sequences demonstrated that the distribution of mtDNA haplotypes is not consistent with a single panmictic unit, and thus suggest the existence of genetically distinguishable sub-populations.

2. METHODS

The samples used in this study are described in Table 1. Total DNA was extracted and PCR amplifications were performed as described by Alvarado Bremer et al. (1996). PCR products were purified for nucleotide sequence analysis using the QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, CA).

The total number of distinct haplotypes was determined using test version 4.0d61 of PAUP, written by David L. Swofford. Neighbor joining (NJ) analyses (Saitou and Nei, 1987) were performed using Tamura-Nei's distances. In cases where there were missing data or insertions and deletions (indels), these sites were ignored in the affected pairwise comparisons. In all of the phylogenetic analyses, trees were rooted using the control region DNA sequences of Pacific northern bluefin tuna (Alvarado Bremer et al., 1997) as the outgroup. Once the total number of haplotypes was established, a minimum spanning network was estimated with the program MINSPAN generously provided by L. Excoffier.

The extent of geographic heterogeneity in allele frequency distribution was analyzed through a Monte Carlo randomization as described in Roff and Bentzen (1989) using the program MONTE in REAP (version 4.) (McElroy et al., 1992). A total of 10,000 randomizations were conducted for each test. We report the probability of obtaining Chi-square value that exceeds or equals the original by chance alone. The inclusion of ties is a conservative approach to handle instances such as the bluefin tuna control region sequence data where many individuals have a unique haplotype.

The level of genetic population differentiation was also estimated using a hierarchical analysis of nucleotide diversity (Holsinger and Mason-Gamer, 1996). This approach provides an analog to Wright's F_{st} to study geographically structured populations using restriction site and DNA data where the variation is not independently inherited. Their measure of g_{st} is used to group populations based on the average time to coalescence for pairs of haplotypes. The results are depicted in a tree diagram that shows the relationship between populations after resampling the data 10,000 times. Significant P-values imply that the mean time to coalescence for two haplotypes drawn from the same node of a tree is less than that for two haplotypes drawn from different nodes.

3. RESULTS AND DISCUSSION

Considerable diversity was observed among the 140 Atlantic northern bluefin tuna mtDNA control region nucleotide sequences. Nearly 60% of the sequences were found only once and the remaining 17 haplotypes were present two to nine times. The probability that two randomly chosen haplotypes from the entire sample differ at any nucleotide is approximately 2.3%. Although this value is higher than that reported by Alvarado Bremer et al. (1997) for a small sample of bluefin tuna, it is considerably lower than the values reported recently for bigeye tuna (7.2%) (Alvarado Bremer et al., 1997; Alvarado Bremer et al., in press) and swordfish (4.6%) (Alvarado Bremer et al., 1996).

Phylogenetic analyses of the mtDNA control region nucleotide sequences resulted in a tree that contains few well supported branches. A total of 99 distinct haplotypes were identified (Table 2). Two of these haplotypes are more closely related to the highly divergent mtDNA of the Pacific northern bluefin tuna. More than half of the haplotypes differ from each other by a single mutation out of a total of 348 bp. The short distances between haplotypes gives rise to a phylogeny with

a star-like appearance (Fig. 1) where most types radiate from a centroid, which in this case is haplotype 36. However, in both the neighbor joining tree (data not shown) and the minimum spanning network, it is apparent that some of the branches contain haplotypes which differ from the rest by several mutational steps. One of these branches, which is supported by bootstrap values higher than 60%, is characterized by three single base pair changes. Members of this group which include haplotypes 1, 3 and 64 and other types radiating from the first two, comprise 31% of the Mediterranean sample, but only 11% of northwestern Atlantic sample. If this frequency difference is temporally stable, then it could demonstrate among-regions heterogeneity.

Before comparing the Mediterranean with the west Atlantic, the stability in allele frequencies was tested within the former since we had replicate sampling years for this region. These samples were: Italy 1993, Italy 1996 and Turkey 1995 all listed in Table 1. The results of Monte Carlo distribution of chi-square values on the pooled analysis of these three Mediterranean samples indicate no heterogeneity among them ($P=0.3721$). Similarly, the binary comparisons of these three samples indicate no difference between any of the sample-pairs ($P>0.4784$). These results suggest that the frequency distribution of haplotypes within the Mediterranean is stable both geographically and temporally. Clearly, additional analyses involving comparisons of fish of the same age from different locations and of different cohorts within the same location would be desirable to validate these findings. Nevertheless, since no differences among Mediterranean samples were detected, these samples were pooled and compared against the sample from the western Atlantic. The chi-square analysis indicates significant differences between these two samples ($P<0.0428$). Thus, these results reject the null hypothesis of homogeneity.

The results from the hierarchical analysis of nucleotide diversity are presented in Fig. 2. Since two of the haplotypes discovered in the Mediterranean sample were more closely related to the Pacific northern bluefin tuna, we decided to pool them with the Pacific bluefin tuna sample also included in the analysis. This procedure is a more conservative approach to test the hypothesis of no difference since keeping the two highly divergent Pacific-like haplotypes with the rest of the Mediterranean sample would result in inflated values of nucleotide diversity for that sample and thus, increase the likelihood for rejection of the null hypothesis.

As expected from the large genetic distances which separates the mtDNA types of the Pacific and the Atlantic northern bluefin tuna (Alvarado Bremer, 1997), the analysis of nucleotide diversity underscores the difference between these two allopatric populations ($P<0.0000$). The comparison between the pooled Mediterranean sample and the sample from the west Atlantic also results in the rejection of the null hypothesis of homogeneity ($P=0.0270$).

In summary, a hierarchical analysis of nucleotide diversity demonstrated that sequences obtained from the northwestern Atlantic sample were more closely related to each other than they were to sequences in the Mediterranean sample and vice versa (Fig. 2). Thus, we conclude that the two samples are not genetically homogeneous. This conclusion was supported by the results of a Chi-square analysis using Monte Carlo randomizations. Taken together these results suggest that the null hypothesis of one panmictic genetic stock of Atlantic northern bluefin tuna should be rejected. However, since only a limited number of samples were analyzed, these data *should not* necessarily be interpreted as supporting the current two stock hypothesis (ICCAT, 1997). More extensive analyses of samples from a variety of locales must be performed to generate an accurate picture of northern bluefin tuna population substructure.

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FIG. 1

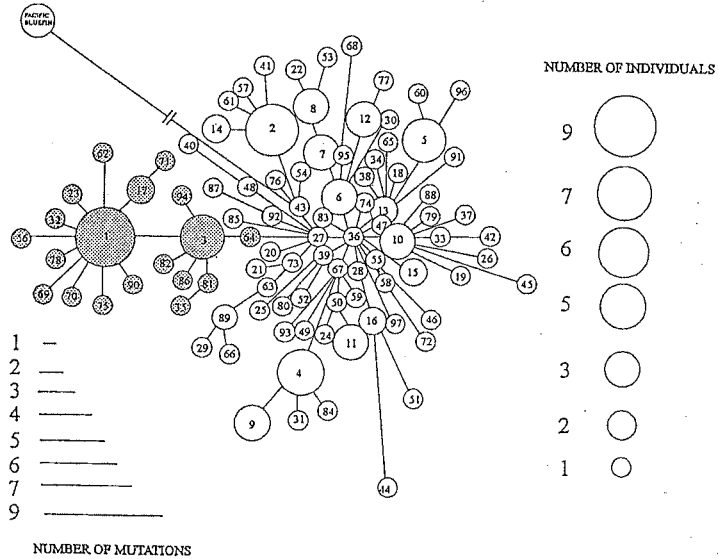


Fig. 1. A minimum spanning network of 99 Atlantic northern bluefin tuna mtDNA haplotypes. Each circle represents an individual haplotype and it is identified by its number. The smallest circle represents one individual and the are of the circles are scaled up proportionally to the number of individuals for a particular haplotype. The length of the branches connecting the network is proportional to the number of steps between haplotypes.

FIG. 2

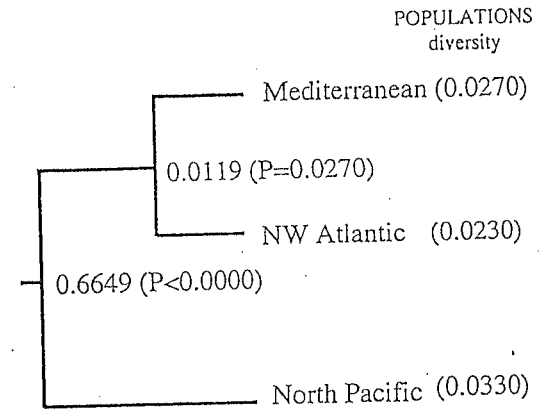


Fig. 2. Tree diagram depicting the relationship between bluefin tuna populations based on the hierarchical analysis of nucleotide diversity. At each node of the tree there is a distance and the probability that the connecting branches are different from each other. The values of nucleotide diversity within populations are given at the tips of the tree.